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Antimicrobials Division (7510P) **Date:** 11/6/08
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DATA EVALUATION RECORD
Executive Summary

STUDY TYPE: Prenatal Developmental Toxicity Study - Rabbit; OPPTS 870.3700b [OPP §83-3b]; OECD 414.

EPA IDENTIFICATION NO.: **EPA PC CODE:**
EPA DP BARCODE:

TEST MATERIAL (PURITY): 80%

SYNONYMS: T99-19

CITATION: MRID 47449001. Senn, C.; Flade, D. (2008) Sanitized T99-19 RGAI (80%): Prenatal Developmental Toxicity Study in the Himalayan Rabbit. Project Number: A92496, 200054704. Unpublished study prepared by RCC, Ltd. 382 p.

EXECUTIVE SUMMARY: T99-19 (80%) was administered by gavage to female Himalayan rabbits on gestation days 6 through 27 at doses of 0, 10, 30, 90 or 180 mg/kg/day. Neither maternal nor developmental toxicity was reported at dose levels of 90 mg/kg or below. At 180 mg/kg/day mean food consumption, mean body weight gain and mean body weight were reduced in maternal animals. At the 180 mg/kg dose level, an increased fetal and litter incidence of fused sternebra were reported and there was a reported increased incidence in the ossification sites in the long bones of the fetal skeletons.

The aternal systemic toxicity No Adverse Effect Level (NOAEL) is 90 mg/kg /day
Maternal systemic toxicity lowest adverse effect level LOAEL is 180 mg/kg/day based on decreases in food consumption, body weight and body weight gain.

The developmental toxicity NOAEL is 90 mg/kg/day
Developmental toxicity LOAEL is 180 mg/kg/day based on increased litter and fetal incidences of fused sternebra and an increased incidence of ossification sites in the long bones.

This study is classified **Acceptable/Guideline** (OPPTS 870.3700b) and as such satisfies the guideline requirements for a developmental toxicity study in the rabbit.

Compliance: A signed and dated Statement of No Data Confidentiality, a Good Laboratory Practice Compliance Statement, a Flagging of Studies for Potential Adverse Effects statement (according to the investigators the study neither meets nor exceeds any of the applicable criteria), and a Quality Assurance Statement were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Sanitized T99-19

Lot/Batch #:	3112
Purity:	80%
Description:	Yellow to brownish wax solid
Reg. #	3090-220
Stability of test compound:	Protected from sunlight, reacts with water.

2. Vehicle and/or positive control: Corn oil

3. Test animals

Species:	Rabbits (113 mated females)
Strain:	Himalayan (SPF)
Age at dosing:	Young adult, 22-31 weeks at pairing
Weight at dosing:	1854-3006 grams
Source:	Charles River Deutschland
Acclimation period:	7 days minimum prior to pairing
Diet:	Pelleted rabbit feed ad libitum
Water:	Tap water ad libitum
Housing:	Individual stainless steel cages

4. Environmental conditions

Temperature:	20 degrees C
Humidity:	30-70%
Air changes:	10-15/hr
Photoperiod:	12/12

B. STUDY DESIGN

1. In-life initiated in February of 2007 and completed in March of 2007

2. Females were placed with males until copulation was observed. The day on which mating occurred was designated as day 0 and females were isolated from the males and caged individually.

3. Animals were assigned using a randomization procedure that was based on body weight. The test material was administered by gavage once a day, beginning on day 6-post coitum and continuing through day 27 post coitum.

Table 1
Study design: Prenatal developmental toxicity test by the oral route in the rabbit

Group No.	Exposure (mg/kg bw/day)	Test formulation concentration (mL/kg)	Number of time-mated females
I	0	1	30
II	10	1	20
III	30	1	20
IV	90	1	20
V	180	1	23

4. Dosing formulations, preparation and analysis: During the first week of the study, samples of the test material were taken and analyzed for concentration, homogeneity and stability. During the last week of the study, samples were evaluated for concentration and homogeneity. Dosing formulations were prepared weekly, as the results of the sample analysis revealed that the test material remained stable for 7 days when stored at room temperature.

5. Statistics: Statistical methodology was used to analyze body weights, food consumption, reproduction and skeletal examination data. Means and standard deviations were routinely calculated throughout the study. Dunnett's t-test was used for intergroup comparisons where appropriate. Steel test was used when data did not follow a normal distribution. Fisher's exact test was also applied when possible.

6. Historical control data

Historical control data were provided for Himalayan rabbits.

C. METHODS

1. Observations for clinical signs of toxicity, including mortality, were conducted at least twice daily. Any animals found dead or that were sacrificed were subjected to a gross macroscopic exam and abnormal tissue was preserved in formaldehyde.

2. Body weights were recorded daily from day 0 until day 28 post-coitum.

3. Food consumption was recorded at three day intervals from day 0 to day 28 post coitum.

4. Sacrifice and pathology

At termination, females were euthanized by an intravenous injection of sodium pentobarbital. A gross external and visceral examination was performed immediately after euthanasia recorded emphasizing any effects observed in the uterus, uterine contents, fetal positions and corpora lutea. Fetuses were removed from the uteri and

killed by subcutaneous injection with sodium pentobarbital. Each fetus was weighed, examined for gross abnormalities. Internal organs were examined and the sex of each fetus was recorded. The cranium was examined for ossification

5. Reproductive outcomes

The following reproductive parameters were evaluated: Pre and post implantation loss, embryonic and fetal deaths, live and dead fetuses, abnormal fetuses, fetal sex ratios and fetal body weights.

6. Evaluation of fetuses

Parameters evaluated in fetuses were body weight and incidences of external/skeletal and visceral/head malformations and variations.

II. RESULTS AND DISCUSSION

A. MATERNAL EFFECTS

1. Clinical signs of toxicity

In the highest dose tested, 180 mg/kg, seven females were observed to have rales or heavy breathing. Three other females experienced gasping and had to be sacrificed.

2. Mortality

Other than the three females who were sacrificed, all others survived until the study was terminated.

3. Body weight, body weight gain and food consumption

In the highest dose treated, the mean food consumption was 24% lower up to day 21 post-coitum. No differences were observed in other dose groups.

Table 3
Prenatal developmental toxicity study in rabbits: Maternal body weight gain/adjusted body weight gain/food consumption

Parameter	Dose (mg/kg bw/day)				
	0	10	30	90	180
Body weight gain 6–28G (g)	210	268	184	189	73
Adjusted[a] body weight gain (%)	-4.7	- 2.0	-4.4	-4.2	- 6.6
Food consumption 6–28G (g/day)	75	88	74	76	57

[a] Weight change using final body weight minus products of conception

4. Reproductive outcomes

At 180 mg/kg, the post implantation loss was significantly increased when compared to controls. This resulted in a decrease in the number of fetuses at this dose level.

Table 4
Prenatal developmental toxicity study in rabbits: Reproductive outcome

Parameter	Dose (mg/kg bw/day)				
	0	10	30	90	180
Number pregnant	30	20	20	20	20
Number aborted/delivered early	1	0	0	0	0
Number killed for ethical reasons	1	0	0	0	3
Number with total resorptions	0	0	0	0	5
Number w implantation sites only	0	0	1	0	0
Number of females w/ live fetuses	28	20	19	20	15
Mean corpora lutea	7.9	7.4	7.7	7.5	6.9
Mean implants/Litter	7.6	7.2	7.2	7.4	6.2
Mean resorptions/Litter (embryonic)	0.3	0.1	0.3	0. 6	1.8*
Mean resorptions/Litter (fetal)	0.1	0.1	0.1	0.1	0.2
Mean live fetuses/Litter	7.2	7.1	6.5	6.8	4.3
Mean dead fetuses/Litter	0	0	0	0	0
Fetal sex ratio[a]	0.54	0.46	0.53	0.35**	0.57
Mean fetal weight (g)	30.9	31.3	31.4	30.3	30.0

[a] Number of male fetuses/total fetuses per litter
* Statistically significant differences at $p < 0.05$., ** statistically significant at $p < 0.01$

5. Gross pathology Five females in the 180 mg/kg dose group had red discolored lungs that was consistent with the test material being inadvertently administered in the respiratory tract or otherwise known as a gavaging error. Other gross findings were sporadic in their occurrence and were considered unrelated to treatment.

B. FETAL EFFECTS

There were no treatment related fetal effects reported for body weights, sex ratios, cranial abnormalities or external and visceral findings. At 180 mg/kg there was an increased incidence of fused and /or wide sternebra. These findings were associated with maternal toxicity and were determined to be "slight" in severity. At this same dose level, there was a reported increase in the level of ossification of the long bones at the highest dose tested; however this increase was not significant when the litters were considered as the unit of measure. There were no treatment related effects on cartilage in the offspring.

III. CONCLUSION

Under the conditions of the study, the maternal and developmental NOAEL for T-99-19 was 90 mg/kg/day. The maternal and developmental LOAEL were both 180 mg/kg/day. The maternal LOAEL was based on a reported decrease in mean food consumption and decreased mean body weight in maternal animals. The maternal observations for food consumption and body weights were statistically significant; however, when corrected body weights were calculated, the body weight gain was similar in all groups. There was also a decrease in the number of fetuses at the 180 mg/kg dose level which was preceded by an increase in the incidence of post implantation loss. The developmental LOAEL was based on an increase in the fetal incidence of ossification sites in the long bones and an increase in both the fetal and litter incidence of fused sternebra.

Sanitized (T-99-19) was not considered to be a developmental toxicant at the doses tested.

IV. REVIEWER'S COMMENTS

A. Maternal toxicity

The maternal NOAEL was 90 mg/kg/day. The maternal LOAEL is 180 mg/kg/day based on a decrease in food consumption, body weight and body weight gain.

B. Developmental toxicity

1. Deaths/Resorptions At 180 mg/kg/day, there was an increase in the number of embryonic resorptions

2. Altered Growth: No effects reported on fetal weight

3. Developmental Variations: No visceral variations were reported which could be attributed to the administration of T99-19

4. Malformations: At 180 mg/kg, there was an increased incidence in fused sternebra (20% for fetal incidence; 60% for litter incidence) when this group was compared to controls (5% for fetal incidence; 29% for litter incidence)

The developmental NOAEL was 90 mg/kg/day. The developmental LOAEL is 180 mg/kg/day based on increased ossification centers in long bones.

While the study author has determined the effect on fetal long bones to be relative to maternal toxicity, it is the opinion of this reviewer that the finding may not have been so much related to maternal toxicity as it may have been related to the age of the fetuses at time of sacrifice. Fetal development at this stage is rapid and a delay in sacrifice of a few hours may lead to more mature fetuses with more defined ossification centers in long bones.

This study is classified **acceptable/guideline** (OPPTS 870.3700b) and satisfies the guideline requirements for a developmental study in the rabbit.